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The Impact of Waste Water Treatments on Seed Germination and Biochemical Parameter of *Abelmoschus Esculentus* L.

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Abstract

A field experiment in green house was conduct to use raw and treated dairy wastewater for watering Abelmoschus esculentus L. (Ladyfinger). In the present study plants were watered using (WW) raw dairy wastewater, (T1) chemicals treatment, (T2) Physical treatment, (T3) dilution method treatment and tap water (TW) in pot experiments. The effect was seen on the germination seed and growth of the plant. The results showed inhibitory effect from dairy effluent (WW) on seed germination and growth plant. Chemicals treatment showed statistically significant differences with other treatments. Treatment (TC2) at 20 Mg/l Al2(SO4)3 and pH 6.5 is improved all growth characters compare with WW and TW reached to 85 %, 70.8 cm, 28.6 cm, 99.4 cm, 65.36 % , 15.86% and 3.543 Mg\g FW for seed germination, shoot length, root length, seedling length, shoot dry matter, root dry matter and chlorophyll respectively. Also concentration 25% and pH 6.5 from dilution method treatment was improved all the qualities but at a lower level. Also maximum favorable effect was observed to (T3) physical treatment ranged from average to moderate in terms of impact. Hence those effluents from dairy. Thus, the dairy effluent, after chemical treatments and proper dilutions can be used as a potential source of water for seed germination and plant growth in agricultural practices.

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Keywords: Treated Dairy Effluent, Physico-chemical properties, Abelmoschus esculentus L Seed germination.

1. INTRODUCTION

Water is one of the most important precious resources found on the earth. The water resources are most often affected by anthropogenic activities and also from industries. Growth of population, massive urbanization, rapid rate of industrialization and modern techniques in agriculture have accelerated the water pollution and led to gradual deterioration of its quality [1], [2]. Due to continuous disposal of waste water into the water bodies, surface water quality throughout the country has deteriorated because of the mixing of various chemical pollutants of the effluent with water [3]. This effluent contains various micro nutrients essential for growth of crop plant. However, many industrial wastes may have harmful effects and may cause soil fatigue [4]. The clean water is used in various stages of dairy operations, such as, milk processing, cleaning, packaging and cleaning of the milk tankers and releases the waste water which is known as dairy effluent. Also high cost and scarcity of chemical fertilizers, the land disposal of agricultural, municipal and industrial waste is widely practiced as a major and economic source of nutrients and organic matter for growing cereal crops [5], [6]. Present study was designed for screening the suitability of dairy effluent to use for irrigation purpose. Crop selected for this purpose was Ladies finger (*Abelmoschus esculentus* L).

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2. MATERIAL AND METHODS

2.1 Experimental procedure: The study was applied in green house at School of Biological Science of Universiti Sains Malaysia (USM), Penang, Malaysia on March to Jun 2012 to study the effect of wastewater (Dairy Effluent) on seed germination and growth of Ladies finger (*Abelmoschus esculentus* L). Maximum temperature during the study period varied between 28-32oC, respectively. Sampling of raw dairy wastewater (WW): The dairy effluent samples were collected in dry plastic containers which are rinsed with HNO3 and distilled water and then in effluent, from Dairy factory, industrial area, Bayan Lepas, 11900, Pulau Pinang, Malaysia. The combined samples of effluent were collected at the main drain pipe which is connected from washing tank to outside discharge unit. Characterization of Dairy Effluent: The physico-chemical properties of dairy effluent samples were analyzed according to standard procedures [2]. The results were given in Table 1. Amelioration of Dairy Effluent, The dairy effluent samples were treated by using different ameliorative techniques physical treatment, chemical coagulation, flocculation and dilution method using different concentrations Table.1. The treated samples were used independently to know their effects on seed germination and other growth parameters of Ladies finger *Abelmoschus esculentus* L. in green house.

2.2 Treated dairy wastewater by different treatment approach

2.2.1 Chemicals treatment (T1): The coagulation flocculation, (Jar test) was used to determined best dose (Alum and polymer) of the jars while mixing using H2SO4 or NaOH /lime. Dose of the selected coagulant Aluminum sulfate Al2(SO4)3 to each jar (15 and 20 Mg/l) and 5 Mg\l Anionic polymer was added to each jar test during test.

2.2.2 Physical treatment (T2): Filtered raw wastewater was obtained. In order to get that, the raw wastewater was filtered through 1µm and 5mµ membrane filters. The samples collected and kept in containers are clean and sterile until use.

2.2.3 Dilution method (T3): The concentrations prepared from mixture raw water with tap water to made dilutions. To preparation of the concentrations 25% and 50 % mixture 1/3 and 2/2 water with raw water in containers and stirred well mixed fully and adjusted the pH before used it directly in the irrigation.

Table 1: Physico-chemical properties of treated Dairy Effluent (WW).

Parameters	Unit	*Standard	Raw dairy w\w	Dilution		Physical treatment		Chemical treatment	
				25%	50%	**1µm	5µm	$Al_2(SO_4)_3$ 15 Mg\l	$Al_2(SO_4)_3$ 20Mg\l
		Values							
pH		5-9	8.570	6.900	7.200	7.300	7.300	6.900	6.500
BOD	mg\l	12	213.490	43.000	100.000	140.000	145.000	20.000	15.000
COD	mg\l	100	505.000	121.000	200.000	470.000	491.000	80.000	71.000
TDS	mg\l	4000	715.760	203.000	400.000	710.000	713.000	307.000	256.000
TSS	mg\l	400	444.980	250.000	350.000	123.000	205.000	119.000	103.000
N	mg\l	25	7.800	2.632	3.213	7.100	7.534	2.978	1.231
P	mg\l	10	11.000	7.498	9.854	10.565	10.590	4.303	3.561
K	mg\l	20	20.000	7.981	11.701	19.122	19.402	6.530	4.602
Ca	mg\l	< 400	83.000	21.874	45.798	82.290	82.780	51.000	47.132
Mg	mg\l	< 60	30.000	9.4789	17.900	29.010	29.430	12.200	9.331
Cl	mg\l	< 350	48.000	29.712	30.000	48.000	48.000	35.000	35.000

*Standard [7]
Note: ** membrane size

2.3 Parameters studies

Ten plants of each treatment /3 replicate have randomly selected and labeled to collecting data. The data was collected sequentially starting with chlorophyll content pre harvest, vegetative qualities Germination (Shoot, root and seedling length) and dry matter qualitative (Shoot and root dry matter) postharvest.

2.3.1 Germination test (%): The germination test was conducted using the paper towel method as prescribed in ISTA rules [8], by providing the optimum conditions for each test of crop. The daily germination counts were made on normal seedlings and total germination was calculated and expressed in percentage:- *GT = [N_T x 100]\ N
*Where: N_T= Number of seedlings emerging on day and N= Day after planting

2.3.2 Chlorophyll content. Mg\g FW (fresh weight): The chlorophyll content was estimated by extracting fresh leaves with 80% acetone and after centrifugation at 8000 rpm for 20min, measuring the colour intensity of the extract at (645 and 663) nm wave lengths by spectrophotometer (Spectra scan UV 2700). The method of [8] was used to calculate the chlorophyll a and chlorophyll b contents.

2.4 STATISTICAL ANALYSIS

The experimental data was subjected to analysis of variance (ANOVA). Significant differences between a values were determined using Duncan's Multiple Range test ($P<0.05$) following ANOVA. Statistical analyses were performed using SPSS [9] (SPSS version 19 Inc., Chicago, and USA, 2012).

3. RESULTS

Water characteristics: The physico-chemical properties of dairy effluent were given in Table 1. The effluent was milky white in colour, turbid, with oil and having stringent with dislike smell. In general the quality of all the three water samples (T1, T2 and T3) fall within permissible limits for irrigation water as per quality standards Table (1) with Superiority significantly for chemical treatment on of the rest treatments (T1 and T2) except tap water (control) that was different than other treatments with respect to all measured parameters. [10], [11]. The chemical treatment (T1) was better per all water properties and showed significant reduction in TDS and BOD compared with untreated (Table1). Also on an average basis in pH has been improved, while Nutrient concentrations and heavy metals in were below the irrigation standards [3].

3.1 Effect the chemical treatments (T1): The treated dairy effluent samples showed favorable effects on seed germination and other growth parameters of Lady finger *Abelmoschus esculentus* L. compare with crude dairy effluent (untreated) and tap water .The result Table 1 showed presence of statistically significant differences in percentage (%) seed germination, seedling growth (cm) and dry matter production(g) depending on the dose used and the pH value .The maximum promoting effect was recorded in the treatment [TC2 ($Al_2(SO_4)_3$ 20 Mg/ l - PH 6.5)] gave the highest averages for all characteristics under study. Then followed by treatment [TC3 ($Al_2(SO_4)^3$ 20 Mg/ l - PH 7.5)] that improved growth plant generally .Table 1. Other chemical treatments showed little statistical significance when compared with water, but outperformed the untreated raw water that showed inhibitory effect of on growth plant.

Table 2. Effect wastewater chemically treated $Al_2(SO_4)_3$ Mg/l at different pH on plant growth characteristic analyzed by average ANOVA

Treatments	Germination %	Seedling growth (cm)			Shoot dry matter (%)	Root dry matter (%)	Chlorophyll content. Mg\g fw
		Shoot length	Root length	Seedling length			
TW [Tap water)	90.00 [a]	38.00 [i]	11.60 [e]	49.93 [i]	43.75 h	10.294 [e]	2.761 b[e]
WW (raw)	30.00 [f]	23.30 [h]	7.30 [f]	30.60 [h]	29.14 i	6.190 [f]	1.109 [d]
TC1- $Al_2(SO_4)_3$ 20 mg/ l - pH 5.5	80.00 [c]	60.50 [c]	21.70 [c]	82.20 [d]	60.80 c	12.40 [d]	2.949 [ab]
TC2 - $Al_2(SO_4)_3$ 20 mg/ l - PH 6.5	85.00 [b]	70.80 [a]	28.60 [a]	99.40 [a]	65.36 a	15.86 [a]	3.543 [a]
TC3- $Al_2(SO_4)_3$ 20 mg/ l - pH 7.5	80.00 [c]	68.00 [b]	25.80 [b]	93.80 [b]	62.50 b	14.25 [b]	3.00 [ab]
TC4 -$Al_2(SO_4)_3$ 15 mg/ l - pH 5.5	70.00 [e]	53.00 [f]	20.00 [d]	73.00 [f]	50.56 f	11.53 [e]	2.13 [c]
TC5 -$Al_2(SO_4)_3$ 15 mg/ ml - PH 6.5	75.00 [d]	58.30 [d]	25.50 [b]	83.80 [c]	55.76 d	14.566 [b]	2.598 [bc]
TC6 -$Al_2(SO_4)_3$ 15 mg/ ml - pH 7.5	70.00 [e]	55.00 [e]	21.50 [c]	76.50 [e]	52.23 e	12.796 [c]	2.254 [c]

*Alphabets in the same column showed non- significant. (ANOVA P<0.05)

3.2 Physical treatments T2: The results showed statistically significant differences between treated water and untreated water (raw dairy wastewater) effect on the plant growth at green house. Table 3 but did not reach to significant limits to compare with tap water, which outperform on all transactions. All the treatments improved properties growth in plant compare with crude wastewater. The maximum promoting effect was recorded at control; also membrane 1 µm / pH .6.5 (TF2) showed significantly outperformed with all other treatments and gave higher average values for all character under study.
Also there was increase in mean of the averages result for used membrane 1 µm- pH 7.5and 5 µm with 6.5 pH (TF3 and TF5), also Maximum favorable effect was observed to other treatments ranged from average to moderate in terms of impact.

Table 3.Effect wastewater physically treated (membrane size /µm) at different pH on plant growth characteristic analyzed by average ANOVA

Treatments	Germination %	Seedling growth(cm)			Shoot dry matter (%)	Root dry matter (%)	Chlorophyll content. Mg\g fw
		Shoot length	Root length	Seedling length			
TW Tap water (control)	90.00 [a]	38.0 [a]	11.60 [a]	49.60 [a]	43.75 [a]	10.29 [a]	2.76 [a]
WW Wastewater (crud)	30.00 [e]	23.30 [h]	6.30 [e]	29.60 [h]	29.14 [e]	6.19 [ed]	1.10 [i]
T2F1 1µm- pH 5.5	65.00 [c]	28.10 [d]	8.00 [dc]	36.10 [e]	32.00 [d]	6.85[c]	1.36 [e]
T2F2 1µm- pH 6.5	70.00 [b]	31.50 [b]	9.23 [b]	40.73 [b]	35.12 [b]	8.00 [b]	1.85 [b]
T2F3 1µm- pH 7.5	65.00 [c]	29.73 [c]	8.73 [bc]	38.46 [c]	33.33 [c]	7.23 [c]	1.55 [c]
T2F4 5µm- pH 5.5	60.00 [b]	25.40 [f]	6.75 [e]	32.15 [f]	31.06 [d]	6.48 [d]	1.21 [h]
T2F5 5µm- pH 6.5	65.00 [c]	29.00 [c]	7.76 [d]	36.76 [e]	33.00 [c]	6.96 [c]	1.43 [d]
T 2 F6 5µm- pH 7.5	60.00 [b]	26.73 [e]	6.88 [e]	33.03 [f]	31.60 [d]	6.53 [c]	1.26 [f]

*Alphabets in the same column show non- significant. (ANOVA P<0.05)

3.3 Dilution method T3: Seedling growth and dry matter production in Lady Finger *Abelmoschus esculentus* L. Results were shown in Table 4 seed germination; seedling growth and dry matter production are significantly differed with different concentrations and pH values of dairy effluent. The maximum promoting effect was recorded

at control and TCO2 (25% -6.5) and TC3 (25% -7.5) Tables 4 and concentration 50% showed less impact in improving the qualities. All the treatments using concentrations showed improve better than tap water and wastewater that was a negative impact on plant. In general the germination (%) and seedling growth decreased with increase in concentration of the dairy effluent. Germination percentage, seedling growth were inhibited at 100 % concentration.

Table 4.Effect of the use of Dilution treatment (%) at different pH on plant growth characteristic analyzed by average ANOVA

Treatments	Germination %	Seedling growth(cm)			Shoot dry matter (%)	Root dry matter (%)	Chlorophyll content. Mg\g fw
		Shoot length	Root length	Seedling length			
Tap water (control)	90.00 [a]	38.00 [f]	11.60 [f]	49.93 [f]	43.75 [e]	9.62 [e]	2.76 [c]
Wastewater (crud)	30.00 [f]	23.30 [h]	7.30 [h]	30.60[h]	29.14 [h]	6.19 [g]	1.10 [f]
TCO1 - 25% - pH 5.5	80.00 [c]	48.50 [c]	14.20 [c]	62.70 [c]	50.11 [c]	11.36 [c]	2.96 [b]
TCO2 - 25% - pH 6.5	85.00 [b]	52.70 [a]	18.60 [a]	71.30 [a]	61.50 [a]	13.30 [a]	3.55 [a]
TCO3 - 25% - pH 7.5	80.00 [c]	50.50 [b]	16.30 [b]	67.46 [b]	54.62 [b]	12.34 [b]	3.09 [b]
TCO4 - 50% - pH 5.5	70.00 [e]	34.10 [g]	10.00 [g]	44.10 [g]	38.00 [g]	8.32 f	1.89 [e]
TCO5 -50% - pH 6.5	75.00 [d]	42.50 [d]	14.10 [d]	56.60 [d]	47.80 [d]	10.76 [d]	2.75 [c]
TCO6 -50% - pH 7.5	70.00 [e]	39.00 [e]	12.40 [e]	51.40 [e]	41.40 [f]	8.67 [f]	2.42 [d]

*Alphabets in the same column show non- significant. (ANOVA P<0.05)

4. DISCUSSIONS

Phytoremediation is an eco-friendly method by which wastewater can be treated to reduce various contaminants which would be otherwise carried into the environment. Different ameliorative techniques improved the quality of effluent by reducing the amount of pollutants present in it. The ameliorative techniques effectively reduced colour, odour, pH, suspended solids and oxygen demanding waste present in the effluent [12], [13].

Generally in this study chemical treatment was improved water properties for germination seed and plant growth these results are consistent with what came in with other study significantly by researcher [14], when study the removal of organic impurities from contaminated wastewater of a dairy plant by coagulation with Aluminum chloride at pH 2-12.

The treated dairy effluent samples using (Potassium alum) at different concentrations [(5-25 Mg/ l) $Al_2(SO_4)_3$] showed favorable effects on seed germination and other growth parameters of Maize at 20 Mg/l , also seed germination, seedling growth and dry matter production are significantly differed in different treatments might be due to presence of less toxic chemicals in the treated effluent and inhibitory effects in raw effluent might be due to presence of high level of toxic substances [15], [13].

Also the results in the Table (1 and 2) consistent with study, [16] found that the treatment of dairy wastewater by coagulation-decantation with iron chloride ($FeCl_3.6\ H_2O$), aluminum sulfate ($Al_2\ (SO_4)_3.18\ H_2O$), and calcium hydroxide ($Ca\ (OH)_2$) removed 40% of organic matter and nitrogen content and improved treated water.

The treated dairy effluent samples using (Potassium alum) at different concentrations [(5-25 Mg/l) $Al_2(SO_4)_3$] showed favorable effects on seed germination and other growth parameters of Maize at 20 mg\l , also seed germination, seedling growth and dry matter production are significantly differed in different treatments might be due to presence of less toxic chemicals in the treated effluent and inhibitory effects in raw effluent might be due to presence of high level of toxic substances [15], [13]. The germination, seedling growth and dry matter production of lady finger *Abelmoschus esculentus* L. are significantly differed in different treatments by using physical treatment Fig 1. The maximum promoting effect was recorded at treatment sand filtrate at 30 minutes [13]. The maximum promoting effect was observed in treated samples might be due to reduction in pollutants in the filters. Germination percentage, seedling growth and dry matter production were inhibited in raw spent wash. It might be due to osmotic pressure caused due to high dose [12]. Osmotic pressure of the spent wash at higher concentrations of total salts

making imbibitions. The result in Table 4 and Fig 3 agreed with study [17] indicated that length of root system and number of lateral roots of Vigna radiata increased by low concentrations of effluent. Similar results had been reported by [18] and [19]. May relate the reduction in seedling (root & shoot) lengths with the elevated amounts of total dissolved solids at higher concentrations. This could also be related to the fact that some of the nutrients present in the effluents are essentials but at high concentration, they become hazardous.

Study of [20] also showed that textile effluents were not inhibitory at low concentrations but with the increase in concentration growth of seedlings was affected. [21] Found that tannery effluents caused a reduction in germination, growth of sunflower parameters along with other parameters like chlorophyll content, protein and carbohydrate content etc.

Effect of fertilizer factory effluents with lower concentrations of effluents promotes the seed germination, seedling growth and chlorophyll content whereas, it was inhibited at higher doses of effluents [22], [13], [1], [23].

Enhancement of chlorophyll could be due to high nutrient uptake, synthesis and translocation probably facilitated by optimum availability of some of the beneficiary plant nutrients and also due to reduction in phenol compounds due to the dilution effect. While, decrease at raw effluent (100%) [12], [24], [15].

5. CONCLUSIONS

The physico-chemical analysis of effluent revealed that it is highly polluted and the quality can be improved by wastewater treatment for recycle and use it in irrigation. *A. esculentus* (ladyfinger) was able to meet its nutrient requirements from wastewater treated and showed established good and enhancing the growth of plants. Chemical treatment using dose of 20 Mg/l $Al_2(SO_4)_3$ with 6.5 and 7.5 pH showed better results in terms of seed germination, seedling growth, dry matter production and biochemical parameters. Hence using raw wastewater treated May have a promising future as a source is safe for irrigation in plantation.

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